INHIBITION OF COLONY-FORMING ABILITY OF BONE MARROW BY SYNGENEIC T LYMPHOCYTES IN AKR MICE WITH LYMPHOBLASTIC LEUKEMIA

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UDC 616.155.392.2-092.9-07:616.419-072.7

KEY WORDS: hematopoietic stem cell; T lymphocyte; leukemia.

The regulatory influence of lymphocytes on the proliferative ability of hematopoietic stem cells is well known [1]. Reports have recently been published of the existence, in certain diseases, of an autoaggressive subpopulation of T lymphocytes which regulates colony formation of bone marrow in vitro. For instance, isolated peripheral blood lymphocytes from patients with various immunodeficiencies and with thymoma [11] and Blackfan–Diamond syndrome [5, 6] inhibit growth of erythroid colonies. In aplastic anemia T lymphocytes can suppress proliferation and differentiation not only of allogeneic, but also of autologous bone marrow cells [3, 8, 9], and in diabetes mellitus [7] and hypogammaglobulinemia they can inactivate normal target cells.

The object of this investigation was to search for a population of lymphocytes which influences the colony-forming ability of bone marrow in spontaneous leukemia developing in AKR mice against the background of thymoma [4]. It was considered important to undertake the investigation by taking into account the age factor, at two distinct periods: preleukemic (from the 21st day after birth until 7 months) and leukemic (8-11 months).

EXPERIMENTAL METHOD

Experiments were carried out on AKR mice obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. So that the work could be done on animals whose ages were exactly known as donors of the interacting cells, mice were crossed and first-generation females were used. The colony-forming

TABLE 1. Interaction between Lymphocytes and Syngeneic Hematopoietic Stem Cells in AKR Mice in the Preleukemic Period (M ± m)

Age of donor mice	Mean No. of colonies after transplantation of $1 \cdot 10^5$ bone marrow cells	Mean No. of colonies after transplantation of bone marrow cells and lymphocytes (1:10)	
		lymph nodes	thymus
21 days	9,0±0,37 (7)	7,4±0,37 (7)	7,4±0,37 (7)
1 months	11,4±0,6 (13)	9,9±0,6 (12)	10,45±0,3 (13)
2 "	9,0±0,4 (7)	9,5±0,6 (7)	10,2±0,5 (7)
3 "	11,6±0,35 (14)	11,25±0,7 (11)	11,5±1,0 (9)
4 "	10,15±0,35 (16)	12,0±0,6 (12)	11,5±1,0 (12)
5 "	7,9±0,5 (9)	6,0±0,5 (7)	6,4±0,4 (10)
6 "	9,3±0,26 (10)	7,75±0,17 (20)	11,3±0,3 (15)
7 "	16,7±1,0 (8)	15,1±1,0 (8)	18,4±1,0 (8)

Legend. Here and in Table 2, number of animals given in parentheses.

Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 717-719, December, 1980. Original article submitted December 29, 1979.

0007-4888/80/9012-1723$07.50

TABLE 2. Interaction between Lymphocytes and Syngeneic Hematopoietic Stem Cells of AKR Mice with Spontaneous Lymphoblastic Leukemia

Mice donating bone marrow	Mean No. of colonies per spleen after transplantation of $1 \cdot 10^5$ bone marrow cells (M ± m)	Ratio between bone marrow cells and lymphocyte	Mean No. of colonies and II after transp. of bone marrow and lymphocytes (M ± m)					
			LLN	II	LT	II	ILN	IT
Leukemic	13,1±0,1 (39)	1 : 1	3,2±0,5 (6)	75	1,2±0,15 (9)	91	9,6±0,3 (7)	15,0±0,6 (6)
		1 : 5	4,35±0,17 (17)	67	2,75±0,16 (13)	79	9,4±0,3 (14)	12,0±0,4 (12)
		1 : 10	2,8±0,07 (18)	79	1,7±0,7 (22)	87	9,8±0,37 (15)	15,1±0,6 (11)
		1 : 20	2,7±0,14 (14)	80	1,2±0,14 (12)	92	10,0±0,25 (13)	11,0±0,25 (19)
Healthy	9,7±0,2 (35)	1 : 1	4,0±0,3 (7)	63	3,9±0,5 (7)	62	10,6±0,4 (7)	14,0±1,3 (6)
		1 : 5	1,5±0,4 (25)	85	2,65±0,13 (24)	73	8,0±0,13 (20)	10,5±0,16 (11)
		1 : 10	1,5±0,14 (15)	85	1,4±0,5 (18)	86	10,7±0,3 (16)	11,0±0,3 (16)
		1 : 20	1,7±0,1 (14)	83	0,0 (8)	100	7,5±0,2 (18)	10,7±0,3 (14)

Legend. LLN) leukemic lymph nodes, LT) leukemic thymus, ILN) intact lymph nodes, IT) intact thymus.

ability of the bone marrow and its dependence on T lymphocytes were studied by the method of Till and McCulloch [10]. Suspensions of bone marrow, lymph node, and thymus cells were prepared from four or five test donors. Bone marrow cells in a dose of $1 \cdot 10^5$, separately or together with lymphocytes or thymocytes in the ratios of 1:1, 1:5, 1:10, and 1:20 were injected intravenously into lethally irradiated (850 R) syngeneic recipients. The mice were killed on the 8th day after transplantation and the extracted spleens were fixed in Bouin's solution; macrocolonies visible to the naked eye were counted, and their number corresponded to the number of colony-forming units. Interaction between cells was assessed by calculation of the inactivation index (II). The diagnosis of leukemia was made intravitally by determination of the morphological composition of the peripheral blood, the leukocyte count, and the hemoglobin concentration. The animals of the experimental group were selected on the basis of these three tests. The results were pooled and subjected to statistical analysis.

EXPERIMENTAL RESULTS

At all ages studied in the preleukemic period no effect of thymus-dependent lymphocytes on colony formation of bone marrow was found (Table 1). However, with the onset and progression of leukemia, diagnosed as lymphoblastic and accompanied by severe anemia (hemoglobin 54-60 units, compared with 100-112 units in the control) and by hypertrophy of the thymus, T lymphocytes began to inhibit hematopoietic stem cells. Colony-forming ability of the bone marrow of both leukemic and healthy mice was sharply reduced in the presence of lymphocytes from leukemic animals: II=62-100% (Table 2). The inactivating ability of lymphocytes and thymocytes was virtually the same and was independent of dose. A dose effect of leukemic thymocytes was exhibited only on healthy bone marrow: with an increase in the dose of thymocytes the percentage of inactivation of colony-forming units increased. The degree of inactivation was the same for both leukemic and healthy bone marrow. Lymphocytes and thymocytes from healthy mice did not change the proliferative activity of the bone marrow of animals of either group.

Interaction between lymphocytes and hematopoietic stem cells, leading to inhibition of hematopoiesis in the bone marrow, can thus take place in AKR mice during the development of lymphoblastic leukemia. It is evidently due, not to a defect of the polypotent stem cell, but either to transformation of thymus-dependent lymphocytes, induced by the leukemogenic virus, and subsequent inhibition of autologous colony-forming units, of the inactivation of allogeneic hematopoietic cells type [2], or to inhibition of the hematopoietic population by a certain humoral factor secreted by lymphocytes.

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IMMUNOLOGIC DETERMINATION OF AN EPIDERMAL G_2-CHALONE-LIKE FACTOR AS A MARKER OF SQUAMOUS-CELL STRUCTURES IN BLADDER TUMORS AND URINE OF RATS

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UDC 616.62-006-008.9:576.353.7]:616.634:576.353.7

KEY WORDS: epidermal G_2-chalone; tumors of the bladder; squamous-cell metaplasia.

One appraoch to the immunodiagnosis of tumors is by the detection, in neoplasms, of products of the tissues whose morphogenetic potential has not developed de novo during neoplastic transformation. For example, the epithelium of the lung and the transitional epithelium of the bladder, both in experimental animals and in man, are sufficiently often affected by metaplastic changes toward stratified squamous (keratinizing) epithelium. It is natural to suggest that such changes will be accompanied by synthesis of tissue-specific products characteristic of keratinizing epithelium.

One of these products is epidermal G_2-chalone, which the authors have isolated from rat skin [3, 6]. This antigenically active glycoprotein, with a molecular weight of about 35,000, can be detected by means of monospecific antiserum only in squamous-cell keratinizing structures. In tissues of the normal lung and unchanged mucosa of the rat urinary bladder this antigen has not been found [1].

Accordingly, in the investigation described below, an attempt was made to discover whether immunologic detection of epidermal G_2-chalone in rat bladder tumors can be used as a diagnostic test. Investigations of this sort were undertaken by the writers previously on induced tumors of the rat lung [2].

EXPERIMENTAL METHOD

Experiments were carried out on 67 male rats, obtained from the "Rappolovo" nursery, weighing 100-120 g or 150-180 g before the experiment. Some of the animals (56 rats) received 0.04% N-nitroso-N-butyl-N-hydroxybutylamine, which induces epithelial tumors of varied histological structure in the bladder of rats after a long period of administration [4, 5], throughout the experiment with their drinking water. The remaining 11 animals were kept on an ordinary diet and served as the control.

The animals were killed 4-11 months after the beginning of exposure and normal or tumor tissue from the urinary bladder was taken for investigation: some of it was used for histological examination (and, in addition, for electron-microscopic study in 20 cases), and some for immunologic study.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 719-721, December, 1980. Original article submitted April 15, 1980.

0007-4888/80/9012-1725$07.50